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# Behavioral Effects of Acute p-Xylene Inhalation in Rats Autoshaping, Motor Activity, and Reversal Learning

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16. ABSTRACT

p-Xylene is a ubiquitous solvent and chemical precursor used in industry, gasoline, and household products. While the population at risk for exposure is thus quite large, little is known about its neurobehavioral effects. To evaluate the possibility that p-xylene affects cognitive behavior, young adult male Long-Evans hooded rats inhaled p-xylene at concentrations of 0 or 1600 pppm, 4 hr per day for 1 to 5 days, and were evaluated after exposure on two learning tasks and a test of motor activity. Autoshaping was carried out across 5 successive days with p-xylene exposure in the morning followed by testing in the afternoon. For this test, the retraction of a single response lever on a variable-time 35-sec schedule predicted delivery of a food pellet. When the force required to depress the lever was 10 g, response acquisition was faster in animals having inhaled 1600 ppm p-xylene than in air-exposed controls. When the force was increased to 20 g, however, p-xylene exposed rats acquired the response no faster than controls. In contrast, inhaled p-xylene at 1600 ppm suppressed response rates in an automaintained reversal learning paradigm without affecting reversal rate. Studies of motor activity showed that while vertically-directed activity was unaffected by p-xylene inhalation, horizontally-directed activity was increased by about 30% for the first 15 min of each daily 25 min test. This p-xylene-induced hyperactivity returned to control levels within each daily test and declined across the 5 daily tests, but was still evident on the fifth day. Rats tested 3.5 hr after termination of p-xylene inhalation were no less hyperactive than rats tested 0.5 hr after exposure. Increased horizontally-directed activity was not correlated across animals with the enhancement of autoshaping. These results were interpreted to indicate that p-xylene inhalation at 16 times the TLV induced hyperactivity and facilitated autoshaping by different means, and that the facilitation of autoshaping was not a directed effect on learning per se.

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# Behavioral Effects of Acute *p*-Xylene Inhalation in Rats: Autoshaping, Motor Activity, and Reversal Learning[1,2]

PHILIP J. BUSHNELL

*Neurotoxicology Division, MD 74B, Health Effects Research Laboratory*
*United States Environmental Protection Agency, Research Triangle Park, NC 27711*

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BUSHNELL, P. J. *Behavioral effects of acute* p*-xylene inhalation in rats: Autoshaping, motor activity, and reversal learning*. NEUROTOXICOL TERATOL **10**(6) 569–577, 1988.—*p*-Xylene is a ubiquitous solvent and chemical precursor used in industry, gasoline, and household products. While the population at risk for exposure is thus quite large, little is known about its neurobehavioral effects. To evaluate the possibility that *p*-xylene affects cognitive behavior, male Long-Evans hooded rats inhaled *p*-xylene at concentrations of 0 or 1600 ppm, 4 hr per day for 1 to 5 days, and were evaluated after exposure on two learning tasks and a test of motor activity. Autoshaping was carried out across 5 successive days with *p*-xylene exposure in the morning followed by testing in the afternoon. For this test, the retraction of a single response lever on a variable-time 35-sec schedule was followed by delivery of a food pellet. When the force required to depress the lever was low (0.10 N), response acquisition was faster in animals having inhaled 1600 ppm *p*-xylene than in air-exposed controls. When the force was increased to 0.20 N, however, *p*-xylene-exposed rats acquired the response no faster than controls. In contrast, inhaled *p*-xylene at 1600 ppm suppressed response rates in an automaintained reversal learning paradigm without affecting reversal rate. Studies of motor activity showed that while vertically-directed activity was unaffected by *p*-xylene, horizontally-directed activity was increased by about 30% for the first 15 min of each daily 25-min test. This *p*-xylene-induced hyperactivity returned to control levels within each daily test and declined across the 5 daily tests, but was still evident on the fifth day. Rats tested 3.5 hr after termination of *p*-xylene inhalation were not less hyperactive than rats tested 0.5 hr after exposure. Increased horizontally-directed activity was not correlated across animals with the enhancement of autoshaping. These results were interpreted to indicate that *p*-xylene inhalation at 16 times the TLV induced hyperactivity and facilitated autoshaping by different means, and that the facilitation of autoshaping involved a change in response topography and not a direct effect on learning ability per se.

Automaintenance Autoshaping Cognitive behavior Motor acitivty Rat Reversal learning Tolerance *p*-Xylene

XYLENE is a volatile organic solvent, the three isomeric forms of which are found in household products, agricultural chemicals, and gasoline (38). *p*-Xylene is used in the synthesis of terephthalate-based polyesters (12) and is thus produced in high quantity [exceeding 4.67 billion pounds in 1986 (39)]. This high production volume coupled with its widespread use results in the potential for extensive public exposure.

The primary route of exposure to xylenes is via inhalation, with dermal absorption being less significant (3, 40, 42, 46). Acute effects of inhaled xylene in humans include irritation of mucosal surfaces (19) and disturbances of postural equilibrium (45) at low concentrations; signs of CNS depression and narcosis become evident at high concentrations (11). Liver and kidney damage (4), and hematological, ophthalmological, and neurophysiological changes may be observed after chronic exposures in animals (10). For airborne xylene, the 8-hr time-weighted average threshold limit value is 100 ppm, and the short-term exposure level is 150 ppm (1).

The acute neurobehavioral effects of inhaled *p*-xylene are typical of those induced by organic solvents in general, and include biphasic changes in motor activity and operant performance, with increased behavior in rodents at low concentrations and decreases at high concentrations [e.g., (6, 21, 27, 30)]. Such effects may impact adversely upon performance during, or immediately following, inhalation of solvents. Therefore the possibility that acute CNS effects of these compounds include changes in learning and memory requires investigation.

Few data regarding effects of xylene on learning and memory have been reported, despite widespread concern

[1]The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

[2]A portion of these data were presented at the Annual Meeting of the Society for Neuroscience, New Orleans, LA, November 18, 1987.

over its role in solvent mixtures producing "chronic toxic encephalopathy" or "organic solvent syndrome" (2) and a report of memory disturbance in histology technicians (23). An early study with rats using an unspecified isomer(s) of xylene showed that daily subcutaneous doses of 0.5 ml/kg/day for 7–28 days retarded learning in a complex maze and caused a 20% reduction in body weight (16). Recent data indicate that neither *p*-xylene nor toluene are neurophysiologically effective by this route, while both solvents affect evoked potentials by the oral and inhalation routes (17). Similar route dependencies were also obtained on measures of motor activity in rats (25). However, ototoxicity has been reported to occur in rats after daily subcutaneous injections of toluene (37). Nevertheless, the changes in body weight make it difficult to interpret the early maze data as a direct effect on learning, and the question of the potential for *p*-xylene to interfere with learning remains open.

Pilot studies in this laboratory indicated that rats emerging from atmospheres of 1600 ppm *p*-xylene showed postural instability, fine tremor, and slightly increased motor activity. Given the lack of information on the cognitive sequelae of acute *p*-xylene inhalation and the concern for humans exposed occupationally to solvents (50), this study was conducted to determine whether *p*-xylene inhalation affected cognitive behavior in rats, and if so, what role might be played by changes in motor activity in those effects. A single exposure concentration of 1600 ppm was used initially to determine whether changes in cognitive behavior would follow *p*-xylene inhalation (Experiment 1). The nature of the change—facilitation of lever press acquisition in an autoshaping paradigm—indicated that the need to determine its behavioral basis outweighed the need to determine its relationship to *p*-xylene concentration; mechanistic rather than concentration-effect studies were thus pursued in Experiments 2 and 3.

## EXPERIMENT 1

Autoshaping (5,20) has been used to quantify the rate at which animals acquire new behavior and to evaluate the effects of CNS lesions (34, 36, 43) and chemical exposures (26, 31, 32). Autoshaping procedures for rats typically involve pairing the retraction of a response lever with delivery of a food pellet. Pairing lever presentation with impending availability of food elicits an increase in physical activity by the rat in close proximity to the lever. This activity may result in either contact with, or—when vigorous enough—movement of, the lever: this movement can then be detected as a response. The initial events in the autoshaping process can be described in Pavlovian terms, with the lever retraction as a CS, the pellet as US, lever response as CR, and retrieval and ingestion of the reinforcer as UR (20).

Autoshaping traditionally involves no instrumental contingency, since the animal's behavior does not affect food delivery at the end of the trial. More typically, however, a combined autoshaping-operant (14) procedure is used, in which the rat's response causes food delivery. As a consequence of the contingency, responding initially elicited by the temporal contiguity of the two stimuli is subsequently maintained by the instrumental contingency between response and reward.

It is important to note that not all activity directed toward the lever will cause its depression; indeed, the topography of the CR often resembles that of the UR (22,35). Thus in many cases, a food US will elicit licking and gnawing of the lever, which may or may not register as a lever press. For this reason, touch-detecting circuits have been used to register responses to the lever (26, 31, 32, 34). On the other hand, requiring a lever press permits one to vary the force necessary to make a response, thus adding a dimension along which the rate of lever press acquisition can be controlled parametrically.

In Experiment 1, rats were trained to lever press using a combined autoshaping-operant technique in a series of daily 1-hr tests following 4-hr exposures to *p*-xylene. To facilitate acquisition of the lever press response, a light lever force was used.

### METHOD

#### *Subjects*

Subjects were 16 male Long-Evans hooded rats, 150 days of age at the time of testing. Animals were housed singly in suspended plastic cages on pine chip bedding, under a 12 hr:12 hr L:D photoperiod with light onset at 0700 hr. All exposures and testing occurred during the light phase of the cycle. Each animal's body weight was maintained at 350 g by scheduled feeding for 2 months prior to and throughout the study. Water was available in the home cage ad lib.

#### *Apparatus*

The apparatus consisted of 4 standard rat operant conditioning chambers (Coulbourn Instruments, Lehigh Valley, PA), each equipped with a single retractable response lever (Gerbrands, Inc., Arlington, MA) 4.8 cm in width, that was flush with the chamber wall when retracted, and protruded 2.0 cm into the chamber when extended. The lever was mounted 4.0 cm from the grid floor in the center of one wall of the chamber. A white light, mounted immediately above the lever, was directed downward on its top surface. A recessed food cup with a swinging plastic door (Campden Instruments, via Stoelting Co., Chicago, IL) was mounted to the right of the lever. A microswitch attached to the door registered nosepokes into the food cup. The levers were adjusted to register depressions of 0.10±0.01 Newtons (N: 0.10 N = 10 g). Each chamber was located in a sound-attenuating shell (Gerbrands, Inc., Arlington, MA) within which white noise (80 dB SPL, Flat, measured at the opening of the food cup) was provided. Reinforcers were 45-mg pellets (Bio-Serv, Inc., Frenchtown, NJ). Control of stimuli and recording of responses were accomplished by computer (PDP8/a: Digital Equipment, Maynard, MA) and SKED interface with SUPERSKED software (State Systems, Inc., Kalamazoo, MI).

#### p-*Xylene Exposures*

*p*-Xylene inhalation occurred in two 11.4 cu ft Rochester-type stainless steel chambers, one for air and one for *p*-xylene. Rats were held in individual mesh cages during exposure. *p*-Xylene vapor was generated by metering liquid *p*-xylene (99.7% purity, Fisher Scientific, Raleigh, NC) into a warmed carrier airstream, which was then mixed with the chamber airstream (flow rate≈4 cfm). The *p*-xylene concentration was stabilized at 1600 ppm prior to introduction of the animals, monitored for hydrocarbons in real time (Beckman Instruments, Los Angeles, CA), and confirmed by GC analysis of grab samples 3 to 6 times during each 4-hr exposure. The rise and fall times for the *p*-xylene concentra-

tion were about 10 min; the asymptotic concentration was within ±10% of the target level. Chamber atmospheres were maintained at 20–23°C and 50–70% relative humidity throughout exposure. Exposures occurred between 0900 and 1300 hr each day for 3 days, with behavioral testing beginning 0.5, 1.5, 2.5, or 3.5 hr after removal from the chambers. Two animals were tested at each time after air or *p*-xylene exposure, for a total of 8 animals per exposure group.

*Behavioral Procedure*

Rats were trained to retrieve food pellets from the food cup prior to *p*-xylene exposure. When each rat consumed 50 "free" pellets within 20 min, *p*-xylene exposures began as described above. On the first day of exposure, pellets were delivered to the food cup on a variable-time 50-sec (range, 30 to 70 sec) schedule. Autoshaping began immediately after 8 consecutive nosepokes with latencies of less than 1.5 sec were emitted. If this criterion were not met within 30 trials, the animal was removed from the chamber and retested on the same schedule the next day. When criterion was met, the next trial began with extension and illumination of the lever for 15 sec (CS); its retraction was followed immediately by pellet delivery (US) on every trial. A lever press (CR) during the 15-sec presentation period resulted in immediate retraction of the lever and pellet delivery (operant contingency). Once a rat met the nosepoke criterion for autoshaping, it was tested only under autoshaping/operant conditions; all autoshaping sessions lasted 50 trials. The variable-time intertrial interval (ITI) averaged 35 sec (range, 15 to 55 sec), during which the lever was retracted and only the houselight was illuminated.

*Statistical Analysis*

Frequencies of lever presses were averaged across animals and ten-trial blocks and subjected to a two-way analysis of variance (ANOVA) with *p*-xylene as a between-subject factor (n=8 per group) and blocks as a repeated measure [General Linear Model (44)]. Corrections for loss of degrees of freedom due to variance-covariance asymmetry in the repeated measures ANOVA were estimated by the method of Greenhouse and Geisser (44). Multiple paired comparisons of mean differences at each trial block were conducted after confirmation of a significant *p*-xylene by blocks interaction ($\alpha$=0.05).

## RESULTS AND DISCUSSION

In confirmation of pilot studies, rats exposed to 1600 ppm *p*-xylene appeared slightly activated, some exhibited fine tremor, and most were unsteady on their feet. These effects largely dissipated within 30 min after termination of exposure. An occasional animal showed signs of excessive grooming, as evidenced by wet fur on its face.

*p*-Xylene did not affect the number of trials required by the rats to reach the nosepoke criterion for pellet retrieval from the food cup prior to autoshaping (control mean±SEM = 59.4±10.0 trials; *p*-xylene mean±SEM = 49.0±8.9 trials). Six of the eight rats in each group emitted 10 lever presses in one or both of the last two 10-trial blocks of the study; the other 4 rats responded fewer than 3 times in any 10-trial block. Acquisition of the lever press response was faster in the *p*-xylene-exposed rats than in the controls (Fig. 1), as confirmed by a significant group by trial-block interaction, $F(14,196)=3.38$, $p<0.034$, and a significantly higher frequency of lever presses in *p*-xylene-exposed rats in blocks 3 through 8. There was no evidence that rats tested soon after removal from the *p*-xylene atmosphere responded differently than rats tested later, but the small number of subjects (n=2 per cell) precluded a systematic test of the effect.

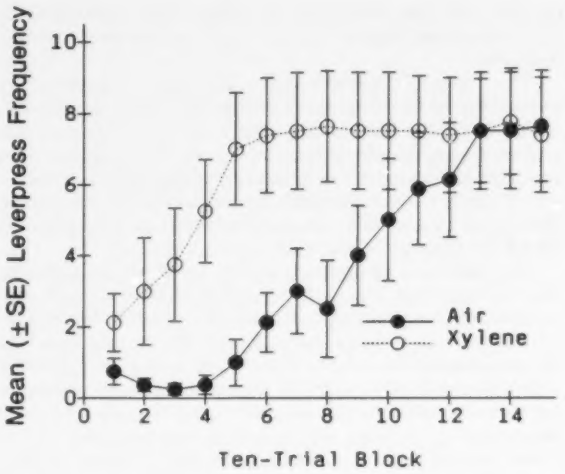


FIG. 1. Mean±SEM frequency of lever presses per 10-trial block of autoshaping for the groups in Experiment 1 exposed either to air (filled circles) or to *p*-xylene (open circles); n=8 per group.

The animals exposed to *p*-xylene increased their frequency of lever pressing faster, and reached asymptote sooner, than controls (Fig. 1). The increase in rate was determined by comparing the slopes of the lines reflecting acquisition of the lever press response for those 12 rats which acquired it. This slope was calculated as the ratio of the change in lever press frequency from the onset of lever pressing (the first of two successive 10-trial blocks with at least 1 lever press) to asymptote (10 lever presses/block), divided by the number of blocks between. Rats having inhaled *p*-xylene produced a steeper slope than did the controls [3.61±0.56 lever presses/block vs. 1.71±0.45 lever presses/block, $t(10)=2.64$, $p<0.05$]. These results indicate that inhalation of *p*-xylene at 1600 ppm facilitated autoshaping. The means by which this unexpected effect was produced were explored in Experiment 2.

## EXPERIMENT 2

One characteristic effect of subnarcotic doses of anesthetics and organic solvents is hyperactivity (6), which may be accompanied by loss of motor control (8). Because there was no a priori reason to expect that *p*-xylene would enhance learning, and because signs of postural instability and tremor were observed in rats immediately after *p*-xylene inhalation, it was hypothesized that the effect in Experiment 1 was due to a disruption of motor control produced by the subnarcotic exposure to *p*-xylene.

To test this hypothesis, two modifications were made to the autoshaping paradigm. If *p*-xylene exposure reduced coordination and fine motor control, then a lever that required a more forceful press would not be as likely to detect an "accidental" response from an intoxicated rat as would a lever requiring less response force. Thus, the force necessary to press the lever was increased to 0.20 N for half the

animals, and the prediction was made that autoshaping in rats trained on "heavy" levers would not be facilitated by *p*-xylene.

The second modification involved reducing the probability of the occurrence of the UCS following the CS, which has been shown to increase the frequency of autoshaped lever presses in rats (15). As this manipulation does not bear any obvious relationship to motor coordination, it was predicted that lever press frequency should increase in all animals trained with reduced probability of reinforcement [P(RFT)], regardless of *p*-xylene exposure.

The influence of general activity levels on autoshaping may be important. Given the tendency for intermediate concentrations of inhaled solvents to increase motor activity (6, 21, 52) and operant response rates (18, 28, 49, 51), the effect on autoshaping observed in Experiment 1 may have resulted from increased motor activity. Motor activity and autoshaping were therefore measured in the same rats after *p*-xylene inhalation, to compare effects on the two measures and to determine the degree to which a change in motor activity could predict the change in autoshaping.

As with Experiment 1, delays of 0.5, 1.5, 2.5, and 3.5 hr intervened between the end of *p*-xylene exposure and the beginning of testing. However, because of the more continuous nature of motor activity data and the larger number of rats tested in Experiment 2, the effect of the delay between *p*-xylene exposure and testing was examined systematically.

Finally, motor activity typically declines across repeated tests in the same test apparatus. This long-term habituation has been taken to reflect a simple, nonassociative learning process [e.g., (48)]. The design of Experiment 2 also permitted evaluation of long-term habituation in rats exposed to *p*-xylene and tested in the same photoactometers on 5 successive days.

## METHOD

### *Subjects*

Subjects were 64 male Long-Evans hooded rats, obtained and housed as in Experiment 1, except that they were 60 or 90 days of age upon arrival in the laboratory, and were maintained at 260 g (60-day rats) or 350 g (90-day rats) by appropriately scheduled feedings.

### *Apparatus*

The apparatus was the same as Experiment 1, with the following exceptions. Half the levers were adjusted to require a depression of at least 0.20±0.01 N to register a response. Motor activity was measured in 4 photoactometers (Motron® Electronic Motility Meter, Stockholm, Sweden) as previously described (24).

### *Procedures*

*p*-Xylene exposures were conducted as in Experiment 1, except that they continued for 5 consecutive days. The experiment was conducted in 4 squads of 16 animals each. Squads 1 and 2 were purchased at 60 days of age, and were used for both autoshaping and motor activity determinations. Squads 3 and 4 were purchased at 90 days of age and were used for autoshaping determinations only. Squads 1 and 3 were trained with P(RFT)=1.0; squads 2 and 4 with P(RFT)=0.8. In each squad, rats were assigned alternately to air and 1600 ppm *p*-xylene conditions, with half the animals in each squad and exposure condition trained on levers requiring 0.10-N response force and the other half 0.20-N response force. In squads 1 and 2, half the rats were tested in autoshaping before motor activity; for the other half, motor activity testing preceded autoshaping. The order of testing was counterbalanced across the other conditions of the experiment, and began 0.5, 1.5, 2.5, or 3.5 hr after termination of exposure to either air or *p*-xylene. During the interval between *p*-xylene exposure and testing, each rat was held individually in a polypropylene transfer cage on bedding material indentical to that used in its home cage.

Autoshaping was conducted as in Experiment 1, except that the combined food-cup training and autoshaping sessions allowed 40 autoshaping trials after reaching the nosepoke response criterion, which was relaxed to require 5 successive nosepokes with latency less than 1.5 sec. Subsequent sessions of autoshaping alone consisted of 50 trials as before. Motor activity was determined in 5 5-min blocks on each of 5 days. Horizontally-directed and vertically-directed behavior were recorded separately for each animal in each time period.

### *Statistical Analyses*

Lever press frequency during the last autoshaping session was analyzed using a three-way ANOVA (44) with *p*-xylene, lever force, and P(RFT) as between-subject variables. Motor activity was analyzed in several ways: first, separate three-way ANOVAs were conducted to evaluate the temporal effects of *p*-xylene on horizontally- and vertically-directed activity, using *p*-xylene as a between-group variable and days and time blocks as repeated measures. Second, the time course of the effect of *p*-xylene on motor activity each day was assessed by examining the interaction between *p*-xylene exposure and the time interval separating the end of exposure and the beginning of the motor activity test. A three-way ANOVA using *p*-xylene and test exposure time as between-group variables and time intervals within a session as a repeated measure was applied to the activity data averaged across test days for this purpose. Third, long-term habituation was evaluated by examining motor activity in the first 5-min block of each daily test across the 5 days of the experiment: a significant *p*-xylene by days interaction would be taken as evidence for an effect of *p*-xylene on this process. In all repeated-measures ANOVAs, the loss of degrees of freedom associated with variance-covariance asymmetry was corrected by the method of Greenhouse and Geisser (44). Finally, the relationship between motor activity and lever press frequency was evaluated by correlating (Pearson's r) the total horizontally-directed activity counts summed across the 5 test days with total lever presses across the 190 autoshaping trials, for the 32 animals (squads 1 and 2) from which both measures were taken.

## RESULTS AND DISCUSSION

The data from the last day of autoshaping (trials 141 to 190) reflected an average of 0.50±0.09 lever presses per trial for the air groups combined and 0.63±0.09 lever presses per trial for the *p*-xylene groups combined, indicating that the shaping process was incomplete, and corresponded in degree of learning to trial blocks 5–10 in Experiment 1. At this stage, the *p*-xylene 0.10-N group emitted more lever presses than either of the air groups or the *p*-xylene 0.20-N group (Fig. 2). The overall analysis of these data showed a significant interaction between *p*-xylene exposure and lever force, $F(1,46)=4.36$, $p<0.04$. By contrast, *p*-xylene exposure did

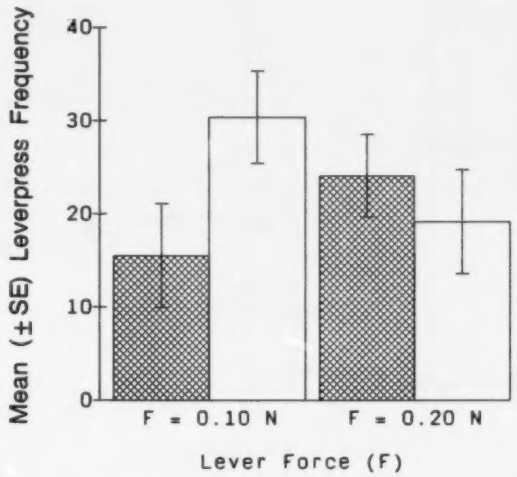


FIG. 2. Mean±SEM frequency of lever presses in the last 40 trials of autoshaping in Experiment 2, as a function of *p*-xylene exposure and lever force; n=13 or 14 per group. Control, hatched bars; *p*-xylene, open bars.

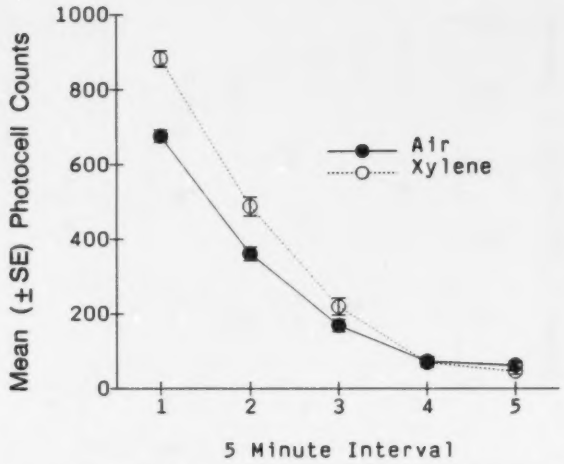


FIG. 3. Mean±SEM frequency of photobeam interruptions in the photoactometer as a function of 5-min interval within a test session, averaged across the 5 successive sessions of Experiment 2; n=16 per group.

not interact with P(RFT), $F(1,46)<1$; nor were significant main effects of *p*-xylene, lever force, P(RFT), or any other interaction between those factors obtained.

Thus the facilitation of autoshaping was observed in Experiment 2 as a systematic replication of Experiment 1, using younger rats and 0.10-N levers. Autoshaping in rats trained with 0.20-N levers was not facilitated, nor was facilitation observed with respect to P(RFT). The specificity of the interaction with lever force indicates that *p*-xylene inhalation modified the rats' behavior toward the levers in a manner which involved a motor component of the lever response.

Exposure to 1600 ppm *p*-xylene increased horizontally-directed activity (Fig. 3), but not vertically-directed activity (data not shown). When averaged across the 5 test days, the increase in horizontally-directed activity induced by *p*-xylene was apparent during the first 15 min of the motor activity test; by 20 min, it was indistinguishable from control. Statistical analysis showed significant main effects of *p*-xylene exposure, $F(1,30)=10.56$, $p<0.003$, days, $F(4,120)=16.78$, $p<0.0001$, and intervals, $F(4,120)=505.47$, $p<0.0001$. In addition, *p*-xylene treatment interacted both with days, $F(4,120)=3.47$, $p<0.01$, and with intervals, $F(4,120)=12.34$, $p<0.0001$; the three-way interaction was also significant, $F(16,480)=2.27$, $p<0.016$. Thus the ability of *p*-xylene to increase horizontally-directed activity declined across both intervals and days, though it remained a significant increase in the first two intervals on day 5 as well [$Fs(1,30)=14.39$, $p<0.0007$ and 7.95, $p<0.008$ for blocks 1 and 2, respectively].

A slight decline in the effect of *p*-xylene on horizontally-directed activity was observed with respect to the time interval between *p*-xylene exposure and testing (Fig. 4). Analysis of these data, averaged across the 5 test days and intervals, showed significant main effects of *p*-xylene, $F(1,24)=15.80$, $p<0.0006$, time interval, $F(3,24)=3.56$, $p<0.029$, and the interaction between *p*-xylene and time interval, $F(3,24)=3.40$, $p<0.034$. The significance of the interaction was due to the lack of effect of *p*-xylene at the 1.5-hr time point, in contrast to the other time points, as reanalysis of these data excluding the 1.5-hr group showed significant effects of *p*-xylene, $F(1,18)=19.16$, $p<0.0004$, and time interval, $F(2,18)=4.12$, $p<0.034$, but not of the interaction between the two factors, $F(2,18)<1$. Thus the effect of *p*-xylene in animals tested 3.5 hr after exposure was not significantly different from the effect of *p*-xylene in animals tested 0.5 hr after exposure, indicating that the effect of *p*-xylene lasted throughout the duration of each daily series of tests and that the time of testing was a relatively unimportant determinant of the effect.

The *p*-xylene-induced increase in horizontally-directed activity during the first 5-min time interval of each daily test did not change significantly across the 5 days of the study (Fig. 5). Statistical analysis showed significant main effects of *p*-xylene, $F(1,30)=28.82$, $p<0.0001$, and days, $F(4,120)=14.34$, $p<0.0001$, without significant interaction between the two factors, $F(4,120)=2.10$, $p<0.09$. In other words, horizontally-directed activity declined in parallel across repeated tests in the photoactometer for rats exposed to both air and *p*-xylene, indicating that the nonassociative learning involved in long-term habituation to the experimental conditions was not affected by *p*-xylene inhalation.

Despite the fact that *p*-xylene increased horizontally-directed activity and lever pressing, the two measures did not correlate across subjects, either across animals trained with 0.10-N lever force ($r=.03$) or across those trained with 0.20-N lever force ($r=.13$). This pattern indicates that those rats whose horizontally-directed activity was increased the most were not the same animals whose lever press frequency was most elevated by *p*-xylene inhalation, regardless of lever force. This lack of correlation suggests that increased motor activity was not related to the *p*-xylene-induced facilitation of autoshaping.

Nevertheless, the fact that the facilitation of autoshaping was blocked by increasing the force requirement on the lever suggests a motor component to the effect of *p*-xylene on autoshaping. Further, the observation of tremor and de-

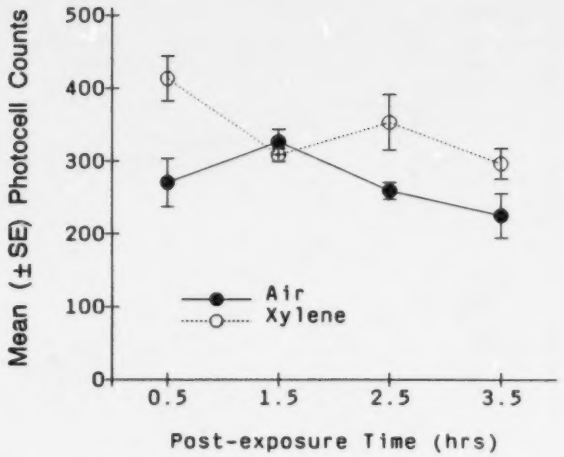


FIG. 4. Mean±SEM frequency of photobeam interruptions in the photoactometer as a function of time after exposure to *p*-xylene in Experiment 2. Data were averaged across time intervals and sessions; n=16 per group.

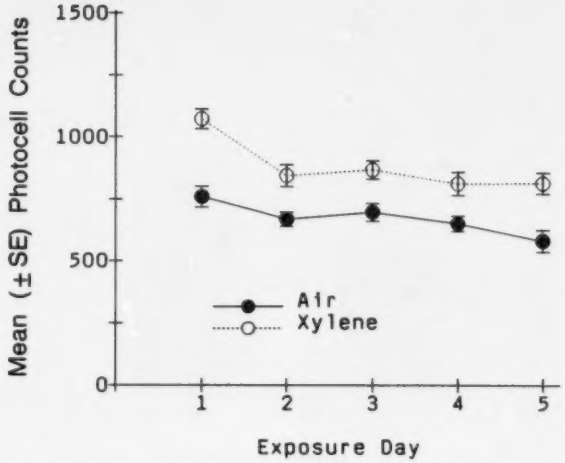


FIG. 5. Mean±SEM frequency of photobeam interruptions in the photoactometer during the first 5-min block only, as a function of day of exposure to *p*-xylene; n=16 per group.

creased steadiness in exposed rats suggests that *p*-xylene degraded control of fine motor movements in these animals. It is therefore likely that *p*-xylene altered the motor responses used by the rat to interact with the response lever. If this alteration then increased the probability that interaction with the lever produced a response, the exposed rats would have encountered the contingency between lever press and reward sooner in the shaping process that did the controls. Given unimpaired recognition of the contingency between the lever press response and immediate pellet delivery, facilitated autoshaping in the *p*-xylene rats would then follow simply from repetition of the lever press response on subsequent trials.

The hypothesis that impaired fine motor control produced the facilitation of autoshaping is based upon observations in a paradigm in which a single, discrete, unpracticed response produced reward on each trial. This hypothesis predicts a different effect of *p*-xylene on well-practiced, high-probability behavior, which should, if anything, be suppressed by impaired motor ability. Experiment 3 was conducted to determine whether *p*-xylene under the same conditions would alter a baseline of relatively high response rates.

## EXPERIMENT 3

Automaintenance describes the process by which a CR is elicited by the pairing of a CS and US (reward) in the Pavlovian sense: the CR is neither necessary nor sufficient for delivery of that reward. Operationally, responses to a lever whose retraction (CS) is paired with food delivery (US) are maintained by the ability of the CS to predict the US (20).

For the present experiment, a second lever was also introduced, whose retraction was not reliably followed by pellet delivery. Under these conditions, rats respond to the first lever ($CS^+$) at a higher rate than to the second lever, whose actions are temporally uncorrelated with pellet delivery, and which may therefore be designated a $CS^\circ$. It is moreover possible to reverse the contingencies between the levers, such that at a given time the $CS^+$ assumes the valence of the $CS^\circ$ (i.e., its retraction no longer predicts food) and the $CS^\circ$ assumes the valence of the $CS^+$. Under these circumstances, the rat's responses shift toward the new $CS^+$ and away from the new $CS^\circ$, resulting in an automaintained reversal (33).

Automaintained reversals may be administered repeatedly; after about 10 reversals, the rats reach an asymptote at which a reversal becomes complete within a 100-trial session (unpublished). At this point, the effect of chemical treatments may be assessed against a reversal baseline, determined both by the rates of responding to each lever separately and by the rate of reversal. The reversal rate can be quantified as the rate of change of the discrimination ratio (i.e., the proportion of total responses directed to the $CS^+$) across trials.

### METHOD

#### *Subjects*

Subjects were 8 male Long-Evans rats, approximately 1 year of age at the time of testing, housed and fed as described above for Experiments 1 and 2.

#### *Apparatus*

The apparatus consisted of 4 standard rat operant conditioning chambers, identical to those used in Experiments 1 and 2, with the following exceptions. Each chamber was equipped with two retractable response levers (Coulbourn Instruments), 3.4 cm in width, mounted with inside edges 13 cm apart on the front wall of the chamber, and sensitive to depressions of 0.25–0.35 N. A cue light was mounted immediately above each lever, and a food cup was centered between the levers.

#### *Behavioral Tests*

Tests were conducted as follows. A session began with a 2-min period of darkness, with no contingencies in effect, after which the first trial was signalled by illumination of the

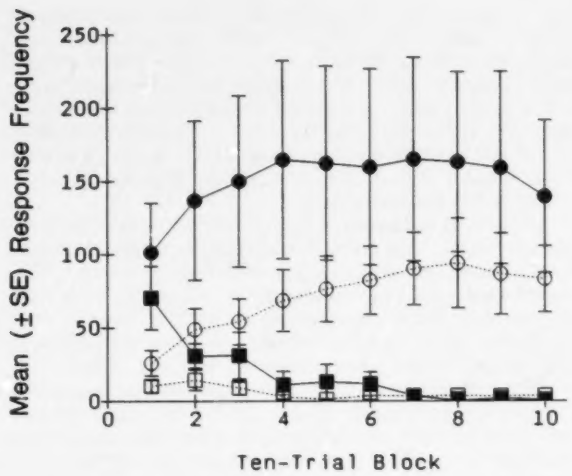


FIG. 6. Mean±SEM (n=8) frequencies of responses to the CS$^+$ (the lever whose retraction was paired with pellet delivery) and to the CS° (the lever whose retraction was temporally uncorrelated with pellet delivery) as a function of both *p*-xylene inhalation and 10-trial block in the reversal learning paradigm of Experiment 3. Responses to the CS$^+$ are shown as circles, those to the CS° as squares; filled symbols represent behavior after air inhalation, open symbols show behavior after *p*-xylene inhalation.

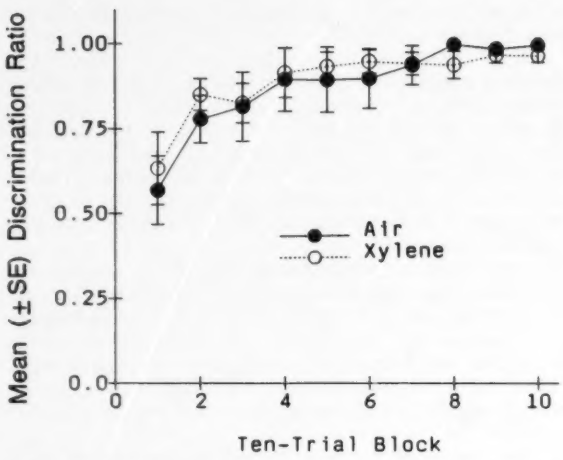


FIG. 7. Mean±SEM (n=8) proportion of the total lever press responses made to the CS$^+$ in Experiment 3, as a function of *p*-xylene exposure and 10-trial block.

houselight. Trials were defined in terms of the schedule by which the lever representing the CS$^+$ was extended and retracted. This lever was extended and its cue light illuminated for a 15-sec period after a variable interval averaging 30 sec (range, 1.6–99.1 sec). It was then retracted, its cue light extinguished, and a pellet was delivered to the food cup with a probability of 0.80. This P(RFT) was chosen to maximize lever press rates at the expense of nosepokes (15). The other lever (CS°) was operated on an identical but temporally independent schedule, which was uncorrelated with pellet delivery. Presses were recorded for each lever separately and summed over 10-trial blocks. Each 100-trial session required about 90 min to complete. Reversals occurred twice per week, on Tuesdays and Thursdays, with the other days serving as nonreversal control days.

### p-*Xylene Exposures*

Exposures were conducted as in Experiments 1 and 2, except 1) each rat was tested following placement in the air chamber for 1 session prior to *p*-xylene exposure (inhalation baseline); 2) half the rats were tested following *p*-xylene inhalation (4 hr × 1600 ppm) and half following air inhalation (4 hr × 0 ppm) on a given reversal day, with exposures switched the following reversal day; and 3) the pair of air-*p*-xylene exposures were administered twice, separated by four days of air exposures. Testing began within 30 minutes of removal from the exposure chambers.

### *Statistical Analysis*

Variability in lever press frequency increased in proportion to absolute response rates, requiring the use of nonparametric tests of the effects of *p*-xylene exposure for the absolute rates of lever pressing. The Wilcoxon signed-ranks test (13) was therefore applied to the air-*p*-xylene difference scores for the 8 animals in the study. Discrimination ratios (DRs) were defined as the frequency of lever presses to CS$^+$ divided by the summed frequencies of lever presses to the CS$^+$ and the CS° within each 10-trial block. DRs were normally distributed, and were thus tested by a two-way ANOVA with *p*-xylene and 10-trial blocks as repeated measures, with Greenhouse-Geisser *df* corrections again applied as needed (44).

## RESULTS AND DISCUSSION

After inhalation of *p*-xylene, responses to both levers were suppressed in frequency (Fig. 6); this change was statistically significant in the first 3 10-trial blocks for responses to the CS$^+$ [Ts(8)=1, 4 and 4, $ps<0.01$, 0.05 and 0.05 for blocks 1–3, respectively]. Responses to CS° were decreased in the first 2 blocks [Ts(8)=1 and 3, $ps<0.01$ and 0.05 for blocks 1 and 2, respectively]. In contrast, the change in DR across trials (Fig. 7) was unchanged by *p*-xylene, $F(1,6)<1$.

These results indicate that *p*-xylene inhalation suppressed responses that were normally emitted at a high rate, without affecting the rat's ability to modify its behavior in response to the contingency reversal. This observation is consistent with the hypothesis that *p*-xylene inhalation disrupted motor control, in that a high-rate response was reduced in frequency. It further suggests that *p*-xylene inhalation suppressed performance (response rate) in the absence of effects on learning.

## GENERAL DISCUSSION

Inhalation of 1600 ppm *p*-xylene for 4 hr facilitated the acquisition of a lever press response in an autoshaping paradigm (Fig. 1). However, this facilitation was eliminated by increasing the force necessary to register each lever press response (Fig. 2), suggesting that it involved a change in motor, rather than cognitive, function. The fact that *p*-xylene treatment did not interact with probability of reinforcement

is consistent with this interpretation, since this manipulation did not require a change in motor behavior from the animals.

The changes in horizontally-directed activity (Figs. 3–5) observed in rats after *p*-xylene inhalation were very similar to those observed previously in mice having inhaled toluene (6) and in rats after inhalation of toluene (52) or *p*-xylene (25), in that horizontally-directed activity was increased at the beginning of each daily test and declined within each test session and across test sessions. In addition, the results of Experiment 2 indicated that the effect of *p*-xylene remained apparent as long as 3.5 hr after *p*-xylene exposure (Fig. 4), and that the decline in initial motor activity across sessions occurred in parallel with controls (Fig. 5). These results indicate that *p*-xylene inhalation produced a complex pattern of change in motor activity characterized by faster within-session habituation and normal long-term (across-session) habituation. Finally, the gradual decline in the hyperactivity across sessions suggests slowly-emerging tolerance to the effects of *p*-xylene (25).

The duration of the effect of *p*-xylene on motor activity is somewhat surprising, given that inhaled *p*-xylene affected visual electrophysiology for just 75 min (17), and given the relatively rapid clearance of *p*-xylene from blood. Most kinetic studies have focused on *m*-xylene, although the three isomers are metabolized quite similarly (46). Clearance of *m*-xylene was described as a three-phased process with half-times of 1–2, 10 and 20 hr, with the slowest phase occuring only after prolonged exposure and likely representing clearance of the compound from adipose tissue (47). Carlsson (9) determined the half-life of inhaled $^{14}$C-labeled *p*-xylene in rat adipose tissue to be 2.2 hr after a 1-hr exposure to 50 ppm *p*-xylene and 6.9 hr after an 8-hr exposure; levels in blood and brain fell more quickly. The present data, showing significant effects of *p*-xylene 3.5 to 4 hr after inhalation, therefore suggest that behavioral changes may persist beyond the expected duration of elevated tissue levels.

In terms of the relationship between motor activity and autoshaping, *p*-xylene produced some activation in the rats, which may have increased the likelihood of their contacting the lever in the autoshaping paradigm and contributed to the facilitation of lever press acquisition observed in Experiment 1. However, the lack of correlation between motor activity and lever press frequency in Experiment 2 suggests that the activation observed in the photoactometer differed from that which enhanced lever press acquisition in autoshaping.

In contrast to its response-enhancing effects in Experiments 1 and 2, *p*-xylene suppressed response rates in Experiment 3. This suppression of high-rate responding is also consistent with an interpretation of *p*-xylene effects reflecting a change in motor control, since incoordination may be expected to reduce the animals' ability to press a lever at a high rate. This pattern may represent a rate-dependent effect of *p*-xylene, akin to the shortening of IRTs by *p*-xylene on a DRL schedule (30) or the reduction in FI quarter-life after exposure to other solvents (29).

Despite the reduction in automaintained lever press rates in Experiment 3, the proportion of total responses emitted to the $CS^+$ was unaffected by *p*-xylene during the reversal. This observation argues against a cognitive effect of *p*-xylene, as the rate of change in responding from the $CS^0$ to the $CS^+$ was unaffected by *p*-xylene inhalation. In addition, long-term habituation to the photoactometer was not affected by *p*-xylene inhalation (Fig. 5), indicating that nonassociative learning was unimpaired. Finally, neither working memory, reference memory, nor learning in a repeated acquisition paradigm were affected by acute exposure to *p*-xylene at the same concentration and duration (7). Taken together, these results indicate that cognitive effects of this exposure to *p*-xylene are unlikely.

In summary, acute inhalation of 1600 ppm *p*-xylene affected behavior in a manner consistent with that of other CNS depressants which, at the lower end of their dose-effect functions, produce excitation and impaired motor control [e.g., (6,8)]. These behavioral changes altered the learning process in the autoshaping paradigm, facilitating acquisition of the lever press response. It is most likely that this change represents a motoric, rather than a cognitive, effect of the solvent, however. This interpretation is consistent with observations of impaired postural equilibrium in humans exposed briefly to 90 ppm *m*-xylene (45). Thus the impact of acute inhalation of *p*-xylene on behavior may include changes in motor control, but are unlikely to involve cognitive capacities per se. The further effects of chronic solvent exposure, if any, remain to be evaluated.

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